In the matter of:
US 10/520,791

I, Dr. Dietmar Forstmeyer,
attorney to BOETERS & LIECK, Oberanger 32, D-80331 München,
Germany, do solemnly and sincerely declare as follows:

1. I am fully conversant with the English and German languages.

2. The attached is a translation which I have made in English of DE 102 30 875.6 which I certify to be a true and correct translation into the English language to the best of my knowledge and belief.

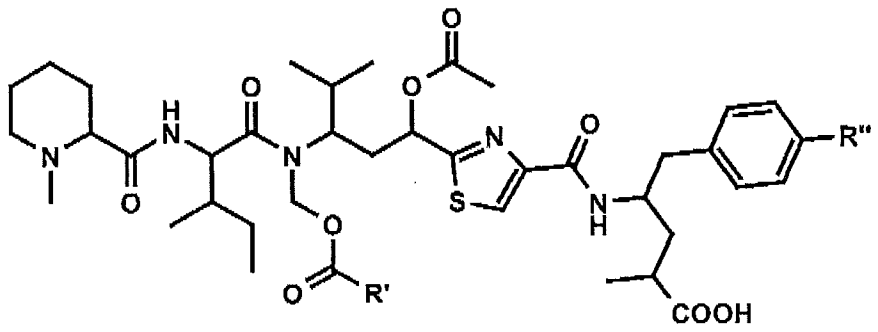
AND I MAKE this solemn declaration, conscientiously believing the same to be true, this 12th day of May 2009.

(Dr. Dietmar Forstmeyer)

Tubulysin bioconjugates

The present invention relates to novel tubulysin bioconjugates, especially antibody conjugates and the use thereof in the treatment of cancer diseases.

The tubulysins were for the first time isolated by the group of Höfle and Reichenbach (GBF Braunschweig) from a culture broth of strains of the Myxobacteria Archangium gephyra (F. Sasse et al. J. Antibiot. 2000, 53, 879-885; WO9813375; DE 10008089). These compounds show an extremely high cytotoxic activity against mammallian cell lines with IC50 values in the picomolar range and therefore, are of high interest as potential therepeutics against cancer.



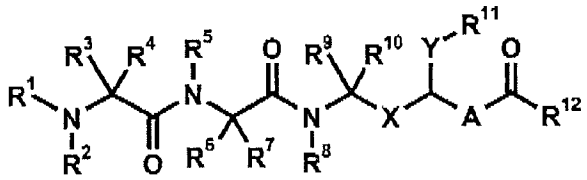
Tubulysin A: R' = $CH_2CH(CH_3)_2$; R'' = OH
Tubulysin B: R' = $CH_2CH_2CH_3$; R'' = OH
Tubulysin C: R' = CH_2CH_3; R'' = OH
Tubulysin D: R' = $CH_2CH(CH_3)_2$; R'' = H
Tubulysin E: R' = $CH_2CH_2CH_3$; R'' = H
Tubulysin F: R' = CH_2CH_3; R'' = H

The extremely high cytotoxicity of tubulysins also exhibits some disadvantages: a high general toxicity as well as a low selectivity against normal cells. One possibility to circumvent these disadvantages is the use of monoclonal antibodies against tumor cell

antigenes as means of transport, which preferably access cancer cells in the body and preserve healthy tissue.

The object of the present invention was to provide novel tubulysins bioconjugates, especially tubulysin antibody conjugates having enhanced pharmacological properties, especially a higher selectivity at an existing cytotoxicity and a lower toxicity when compared with the natural products.

The present invention provides bioconjugates of the general formula U-V-W,
wherein U is represented by formula (I),



(I)

wherein
A is a optionally substituted five or six membered heteroaromatic group;
X is an oxygen atom, a sulfur atom, a group of the formula NR^{13} or $CR^{14}R^{15}$;
Y is an oxygen atom, a sulfur atom or a group of the formula NR^{16} and
the residues R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15} and R^{16} independently from each other are a hydrogen atom, a alkyl-, alkenyl-, alkynyl-, heteroalkyl-, aryl-, heteroaryl-, cycloalkyl-, alkylcycloalkyl-, heteroalkylcycloalkyl-, heterocycloalkyl-, arylalkyl-, or heteroarylalkyl-group or two of the residues together are part of a cycloalkyl- or heterocycloalkyl ring system,
V is a linker and W is a biomolecule.

The term alkyl refers to a saturated straight or branched chain hydrocarbon group, containing from one to 50 carbon atoms, preferably from one to twelve carbon atoms, especially preferred from one to six carbon atoms, for example the methyl, ethyl, iso-propyl, iso-butyl, tert.-butyl, n-hexyl, 2,2-dimethylbutyl, n-octyl, allyl, isoprenyl or hex-2-enyl group.

The terms alkenyl and alkinyl refer to at least partly unsaturated straight or branched chain hydrocarbon groups, containing from two to 50 carbon atoms, preferably from two to twelve carbon atoms, especially preferred from two to six carbon atoms, for example the allyl, acetylenyl, propargyl, isoprenyl or hexa-2-enyl group.

The term heteroalkyl refers to an alkyl, a alkenyl, or a alkynyl group wherein one or more (preferably 1, 2, 3, 4 or 5) carbon atoms are replaced by an oxygen, nitrogen, phosphorous, or sulphur atom (preferably oxygen or nitrogen), e.g. an alkyloxy group such as methoxy or ethoxy, or a methoxymethyl, nitrile, methylcarboxyalkylester, carboxyalkylester or 2,3-dioxyethyl group. The term heteroalkyl furthermore refers to a caboxylic acid or a group derived from a carboxylic acid such as for example acyl, acyloxy, carboxyalkyl, carboxyalkylester e.g. methyl carboxyalkylester, carboxyalkylamide, alkoxycarbonyl or alkoxycarbonyloxy.

The term cycloalkyl and cyclo, resp., refers to a saturated or partially unsaturated cyclic group which comprises one or more rings which build a scaffold, which contains three to fourteen carbon atoms, preferably three to ten carbon atoms, e.g. the cyclopropyl, cyclohexyl, tetralin or cyclohex-2-enyl group.

The term heterocycloalkyl and heterocyclo, resp., refers to a cycloalkyl group as defined above in which one or more (preferably 1, 2 or 3) carbon atoms are replaced by an oxygen, nitrogen, phosphorous or sulfur atom and may e.g. stand for the piperidine, morpholine, N-methyl piperazine, or N-phenyl piperazine group.

The term alkylcycloalkyl and heteroalkylcycloalkyl, resp., refers to groups which according to the definitions given above contain both cycloalkyl or heterocycloalkyl as well as alky, alkenyl, alkynyl and/or heteroalkyl groups.

The term aryl and Ar, resp., refer to a aromatic group which has one or more rings and which is built by a scaffold which contains 5 to 14 carbon atoms, preferably 5 or 6 to 10 carbon

atoms, e.g. a phenyl, naphthyl, 2-, 3- or 4-methoxyphenyl, 2-, 3- or 4-ethoxyphenyl, 4-carboxyphenylalkyl or 4-hydroxyphenyl group.

The term heteroaryl and heteroaromatic, resp., refers to a aryl group in which one or more (preferably 1, 2 or 3) carbon atoms are replaced by an oxygen, nitrogen, phosphorous or sulfur atom, e.g. the 4-pyridyl, 2-imidazolyl, 3-pyrazolyl and isochinolinyl group.

The terms aralkyl and heteroaralkyl, resp., refer to groups which according to the above definitions contain both aryl or heteroaryl, resp., as well as alkyl, alkenyl, alkynyl and/or heteroalkyl and/or cycloalkyl and/or heterocycloalkyl groups, e.g. the tetrahydroisochinolinyl, benzyl, 2- or 3-ethylindolyl or 4-methylpyridino group.

The terms alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, aralkyl and heteroaralkyl also refer to groups in which one or more hydrogen atoms of such groups are replaced by fluoro, chloro, bromo or iodo atoms or OH, SH, NH_2 or NO_2 groups. These terms furthermore refer to groups which are substituted with unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, aralkyl or heteroaralkyl groups.

The term linker refers to a group which is used to connect compounds of the formula (I) with a biomolecule. A linker can be a direct bond, a alkylene, alkenylen, alkynylene heteroalkylene, arylene, heteroarylene, cycloalkylene, alkylcycloalkylene, heteroalkylcycloalkylene, heterocycloalkylene, aralkylene or a heteroaralkylene group. Preferably, the linker is metabolically cleavable. Especially preferably, the linker contains one or more hydrazone and/or disulfide groups.

As biomolecules e.g. glycoproteins, lipoproteins, lectins, hormones, albumine, liposomes, DNA, dextrane, biotin, streptavidine, avidine, cells or antibodies come into question. Preferably the biomolecule is an antibody, especially preferred a monoclonal antibody.

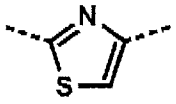
Further examples for linkers and biomolecules are described in G.T. Hermanson, Biokonjugate Techniques, Academic Press, San Diego, 1996.

Due to their substitution, compounds of formula (I) may contain one or more centers of chirality. Therefore, the present invention relates to either all possible pure enantiomers and all possible pure diastereoisomers as well as their mixtures in every mixing ratio.

Preferred X is a CH_2 group.

Moreover preferred Y is an oxygen atom.

Further preferred A has the following structure:



Furthermore preferred R^1 and R^3 are together part of a cycloalkyl ring; more preferred R^1 and R^3 together are of the formula $-(CH_2)_4-$.

Further preferred R^2 is a C_1-C_4 alkyl group, more preferred a methyl group.

Furthermore preferred R^4, R^5, R^6 and R^{10} are hydrogen atoms.

Moreover preferred R^7 is a alkyl group; more preferred a group of the formula $-CH(CH_3)CH_2CH_3$.

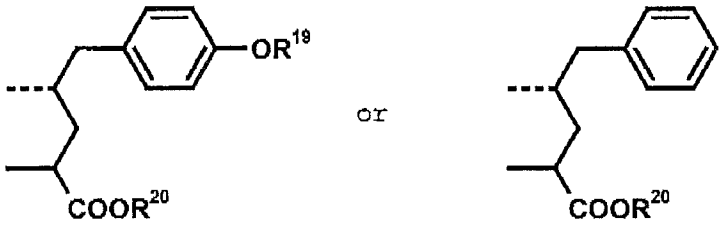
Furthermore preferred R^8 is a hydrogen atom, an alkyl, alkenyl or a heteroalkyl group; more preferred a group of the formula $-CH_2OC(=O)R^{17}$,whereby R^{17} is a C_1-C_6 alkyl or a C_1-C_6 alkenyl group.

Moreover preferred R^9 is a alkyl group; especially preferred a group of the formula $-CH(CH_3)_2$.

Further preferred R^{11} is a hydrogen atom or a acetyl group.

Further preferred R^{12} is a group of the formula NHR^{18} whereby R^{18} is a heteroaralkyl group.

More preferred R^{18} has the following structures:



wherein R^{19} and R^{20} independentely from each other are hydrogen atoms, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, alkylcycloalkyl, heteroalkylcycloalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl groups; especially preferred, R^{19} and R^{20} are hydrogen atoms.

More preferred the compound of formula (I) is Tubulysin A.

Further preferred is the linker connected to compounds of formula (I) via residues R^8, R^{11}, R^{19} or R^{20}; more preferred via R^{19} or R^{20}.

Pharmacologically acceptable salts, solvates, hydrates or formulations of the herein described bioconjugates are also part of the present invention. Examples for pharmacologically acceptable salts of compounds of the formula (I) are salts of physiologically acceptable mineralic acids such as hydrochloric acid, sulfuric acid and phosphoric acid or salts of organic acids such as methansulfonic acid, p-toluenesulfonic acid, lactic acid, acetic acid, formic acid, trifluoroacetic acid, citric acid, succinic acid, fumaric acid, maleinic acid and salicylic acid. Compounds of the formula (I) can be solvated, especially hydrated. The hydratisation can occur e.g. during the preparation process or as consequence of the hygroscopic nature of the initially water free compounds of the formula (I).

The pharmaceutical compositions according to the present invention contain at least one compound of formula (I) as active ingredient and optionally carriers and/or adjuvants.

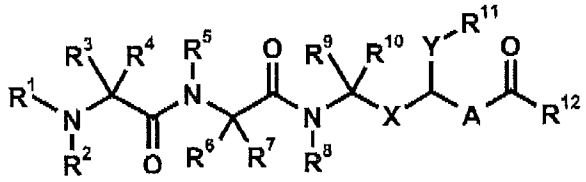
The therapeutic use of compounds of formula (I), their pharmacologically acceptable salts and solvates, resp. and hydrates as well as formulations and pharmaceutical compositions is also part of the present invention.

The use of these active ingredients for the manufacturing of drugs for the treatment of cancer diseases is also part of the present invention. Furthermore, the present compounds are of great interest for the prevention and/or treatment of rheumatoid arthritis, inflammatory diseases, immunologically caused diseases (e.g. diabetes type 1), autoimmuno diseases as well as further tumor diseases. In general compounds of formula (I) will be administered by using the known and acceptable modes known in the art, either alone or in combination with any other therapeutic agent. Such therapeutically useful agents can be administered preferably parenteral, e.g. as injectable solution. For the production of liquid solutions one may use excipients as are e.g. water, alcohols or aqueous saline. The pharmaceutically useful agents may also contain additives for conservation, stabilisation, salts to change the osmotic pressure, buffers and antioxidants.

Combinations with other therapeutic agents may contain further active ingredients which are usually employed in the therapy of cancer diseases.

Patent Claims

1. Bioconjugates of general formula U-V-W, wherein U refers to the formula (I),



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wherein
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X is an oxygen atom, a sulfur atom, a group of the formula NR^{13} or $CR^{14}R^{15}$;
Y is an oxygen atom, a sulfur atom or a group of the formula NR^{16} and
the residues R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15} and R^{16} independently from each other are a hydrogen atom, an alkyl-, alkenyl-, alkynyl-, heteroalkyl-, aryl-, heteroaryl-, cycloalkyl-, alkylcycloalkyl-, heteroalkylcycloalkyl-, heterocycloalkyl-, arylalkyl-, or heteroarylalkyl-group or two of the residues together are part of a cycloalkyl- or heterocycloalkyl ring system,
V is a linker and W is a biomolecule.

2. Bioconjugates according to claim 1, wherein the compound of formula (I) is Tubulysin A.

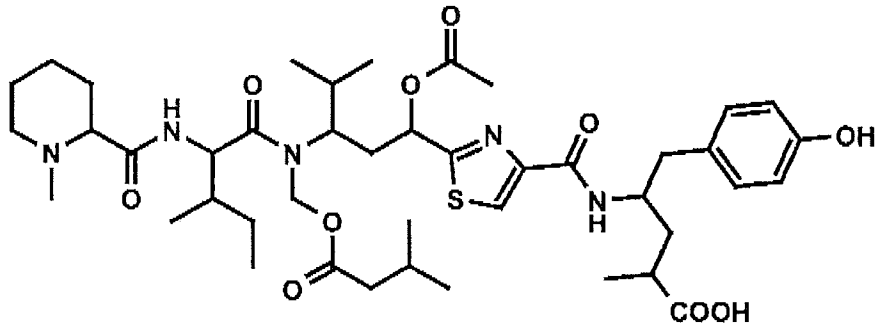
3. Bioconjugates according to claim 1 or 2, wherein the biomolecule is an antibody.

4. Bioconjugates according to claim 1 or 2, wherein the biomolecule is a monoclonal antibody.

5. Use of a bioconjugate according to one of claims 1 to 4 for the treatment of cancer diseases.

Abstract

The resent invention relates to novel tubulysin bioconjugates, especially antibody conjugates (e.g. of tubulysin A) and the use thereof in the treatment of cancer diseases.



Tubulysin A